PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 June 2000 (26.06.00)	
International application No. PCT/NL99/00755	**Applicant's or agent's file reference** BO 42260
International filing date (day/month/year) 09 December 1999 (09.12.99)	**Priority date** (day/month/year) 09 December 1998 (09.12.98)
Applicant DE JONG, Patricia et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 May 2000 (03.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

09/857796

WO 00/33854
PCT/NL99/00755

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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To:
DE BRUIJN, Leendert C.
Nederlandsch Octrooibureau
Scheveningseweg 82, P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 15 June 2000 (15.06.00)	
Applicant's or agent's file reference BO 42260	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/NL99/00755	09 December 1999 (09.12.99)	09 December 1998 (09.12.98)

Applicant
N.V. NUTRICIA et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   15 June 2000 (15.06.00) under No. WO 00/33854

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

DE BRUIJN, Leendert C.
Nederlandsch Octrooibureau
Scheveningseweg 82, P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 10 February 2000 (10.02.00)	
Applicant's or agent's file reference BO 42260	**IMPORTANT NOTIFICATION**
International application No. PCT/NL99/00755	International filing date (day/month/year) 09 December 1999 (09.12.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 09 December 1998 (09.12.98)
Applicant N.V. NUTRICIA et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
09 Dece 1998 (09.12.98)	1010770	NL	23 Dece 1999 (23.12.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Marc Salzman

Telephone No. (41-22) 338.83.38

CLAIMS

1. Preparation having a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, which contains one or more probiotics and one or more non-digestible oligosaccharides.

2. Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerisation of 2 to 20, preferably of 2 to 10, more preferentially of 2 to 6.

3. Preparation according to Claim 1 or 2, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.

4. Preparation according to Claim 3, wherein the polysaccharides are chosen from β-(arabino)galactans, β-mannans, xylans and combinations thereof.

5. Preparation according to Claim 1 or 2, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

6. Preparation according to one of the preceding claims, wherein the probiotics comprise at least one bacterial strain or at least one yeast strain, or a combination of at least one bacterial strain and at least one yeast strain.

7. Preparation according to one of the preceding claims, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lactobacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a *Saccharomyces* species.

8. Preparation according to one of the preceding claims which also contains dead yeast cells.

9. Preparation according to one of the preceding claims, wherein the ratio between the

one or more probiotics and the one or more non-digestible oligosaccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

10. Preparation according to one of the preceding claims which contains the probiotics in a concentration of 10^7 to 10^{11} live cells per gram of total product.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BO 42260		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL99/00755	09/12/1999	09/12/1998
International Patent Classification (IPC) or national classification and IPC A61K35/74		
Applicant N.V. NUTRICIA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/05/2000	23.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Laffargue-Haak, T Telephone No. +49 89 2399 8009

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00755

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-9	as originally filed

Claims, No.:

1-9	as received on	27/11/2000	with letter of	27/11/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-5, 7, 8
	No:	Claims	1,2, 6, 9
Inventive step (IS)	Yes:	Claims	--
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	---

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

Novelty

D4 (WO 98/26787) discloses a method of enhancing a resident population of micro-organisms in the GI tract, based on the administration of beta-glucan (originating from oats, barley, yeast and bacteria, see p. 4, l. 25-35), oligosaccharides (p. 4, l. 5-8) and probiotic micro-organisms (*L. fermentum, Saccharomyces* sp. ; p. 5, l. 9-15). D4 anticipates the subject-matter of claims 1, 2, 6 and 9 as independent claim 1 of the present application relates to preparations *containing* probiotics ... and ... oligosaccharides. The scope of this claims also includes preparations consisting of probiotics, oligosaccharides and other substances (i.e. polysaccharides such as beta-glucan). In addition, claims 1 and 2 are also considered to includes non-digestable polysaccharides (see Section VIII).

Inventive step

D4, considered as the closest prior art, differs from the present application by the use of one or more specific non-digestible oligosaccharides. The technical problem underlying the present invention is to provide alternative synbiotic compositions. The specific non-digestible oligosaccharides are disclosed in D1 (p. 5, l. 32-38) and claims 3-5 and 8 lack an inventive step in the light of D4+D1. The addition of dead yeast cells is known from D3 (p.3, l. 7-11) and the subject-matter of claim 7 lacks an inventive step in the light of D4+D1.

Alternatively, D1 could be considered as the closest prior art and the difference would be the inclusion of yeast. The technical effect associated with yeast seems to be to supply mannoproteins which are believed to prevent the adhesion of bacteria to the intestinal wall (see present description p. 4, l. 11-13). It is known from D3 (p. 2, l. 10-29) and D4 (p. 2, l. 4-12) that mannans (or mannoproteins) and glucans are always present in cell walls, including in yeast cell walls. It is also known that mannans, glucans and fructans competitively inhibit the adhesion sites used by pathogens (see D3). It is therefore considered obvious to combine the teachings of D3-D4 with D1 in order to solve the technical problem.

VI Certain documents cited

In view of the unavailibility of the present priority documents it has not been possible for the IPEA to establish if the present claims are entitled to their earliest declared priority date. The present assessment of novelty and inventive step has been made on the assumption that the claims **are** intitled to their earliest priority date. The following document may however be considered to be relevant according to R. 70.10 EPC :

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
Present application	---	*09.12.1999*	*09.12.1998*
EP 904 784 (= D2)	31.03.1999	22.09.1997	not applicable

VII Certain defects in the international application

It is suggested to add the publication number of D2 in the description on p. 3, 5 and 6 (EP97202900.3 = EP 904 784).

The description is not in conformity with the *amended* claims as required by Rule 5.1(a)(iii) PCT. In particular, only Example II appears to fall within the scope of present independent claim 1.

VIII Certain observations on the international application

The scope of claims 1 and 2 is unclear as oligosaccharides having a degree of polymerisation of 2 to 20 are within the scope of the claims. Oligosaccharides are defined as having a 2 to 10 sugar-units and consequently, the scope of present claims 1 and 2 also includes polysaccharides (i.e. with a polymerisation degree of more than 10).

PATENT COOPERATION TREATY

09/857796

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BO 42260		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 99/00755	09/12/1999	09/12/1998
Applicant		
N.V. NUTRICIA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. ___

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

FOR THE PURPOSES OF INFORMATION ONLY

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09/857796

PCT

REQUEST

17.01.00

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/NL 99/00753
International Application No.

09 DEC 1999 (09.12.99)
International Filing Date

BUREAU VOOR DE INDUSTRIËLE EIGENDOM
P.C.T. INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) BO 42260

Box No. I TITLE OF INVENTION

Preparation that contains oligosaccharides and probiotics

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

N.V. Nutricia
P.O. Box 1
NL-2700 MA ZOETERMEER
The Netherlands

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality:
the Netherlands (NL)

State *(that is, country)* of residence:
the Netherlands (NL)

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

DE JONG, Patricia
Bolderik 3
NL-6026 AM MAARHEEZE
the Netherlands

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below)*

State *(that is, country)* of nationality:
The Netherlands (NL)

State *(that is, country)* of residence:
The Netherlands (NL)

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DE BRUIJN, Leendert C. et al
Nederlandsch Octrooibureau
Scheveningseweg 82, P.O. Box 29720
2502 LS Den Haag
NETHERLANDS

Telephone No
(31-70) 350 2300

Facsimile No
(31-70) 350 2328

Teleprinter No

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request for

Sheet No. 2.....

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VAN LAERE, Katrien
Kamperfoeliestraat 11
NL-6666 WS HETEREN
the Netherlands

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Belgium (BE)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ■ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ■ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ■ **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ■ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ■ **AE** United Arab Emirates
- ■ **AL** Albania .....
- ■ **AM** Armenia .....
- ■ **AT** Austria .....
- ■ **AU** Australia .....
- ■ **AZ** Azerbaijan
- ■ **BA** Bosnia and Herzegovina .....
- ■ **BB** Barbados
- ■ **BG** Bulgaria .....
- ■ **BR** Brazil .....
- ■ **BY** Belarus .....
- ■ **CA** Canada
- ■ **CH and LI** Switzerland and Liechtenstein
- ■ **CN** China .....
- ■ **CU** Cuba .....
- ■ **CZ** Czech Republic .....
- ■ **DE** Germany .....
- ■ **DK** Denmark .....
- ■ **EE** Estonia .....
- ■ **ES** Spain .....
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- ■ **GH** Ghana .....
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- ■ **ID** Indonesia
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- ■ **IS** Iceland
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- ■ **KG** Kyrgyzstan .....
- ■ **KP** Democratic People's Republic of Korea .....
- ■ **KR** Republic of Korea .....
- ■ **KZ** Kazakhstan .....
- ■ **LC** Saint Lucia
- ■ **LK** Sri Lanka
- ■ **LR** Liberia
- ■ **LS** Lesotho .....
- ■ **LT** Lithuania
- ■ **LU** Luxembourg
- ■ **LV** Latvia
- ■ **MD** Republic of Moldova .....
- ■ **MG** Madagascar .....
- ■ **MK** The former Yugoslav Republic of Macedonia .....
- ■ **MN** Mongolia
- ■ **MW** Malawi .....
- ■ **MX** Mexico .....
- ■ **NO** Norway
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See Notes to the request form

Box No. VI PRIORITY CL...M ☐ Further priority cla... are indicated in the Supplemental Box.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (09.12.98) 9 December 1998	1010770	the Netherlands		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EPA

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)*	Number	Country *(or regional Office)*
27 August 1999	SN 32349	NL

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 4
description (excluding sequence listing part)	: 10
claims	: 2
abstract	: 1
drawings	:
sequence listing part of description	:
Total number of sheets	: 17

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Search report

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

KRAAG, F.

Nederlandsch Octrooibureau, The Hague, 9 December 1999

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1. Date of actual receipt of the purported international application:	09 DEC 1999 (09.12.99)
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

2. Drawings: ☐ received: ☐ not received:

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Date of receipt of the record copy by the International Bureau: 20 JANUARY 2000 (20.01.00)

Preparaat dat oligosacchariden en probiotica bevat

De onderhavige uitvinding heeft betrekking op een preparaat met gezondheidbevorderende werking, in het bijzonder voor het voorkomen en/of behandelen van aandoeningen van het spijsverteringskanaal, meer in het bijzonder van de darm.

De aanvrage heeft in het bijzonder betrekking op een dergelijk preparaat dat probiotica en onverteerbare oligosacchariden bevat.

Het is bekend dat bepaalde micro-organismen zowel een profylactisch als therapeutisch effect op darmziekten zoals darminfecties hebben. Wanneer deze micro-organismen worden toegediend aan mensen of dieren zullen zij met pathogene bacteriën concurreren om voedingsstoffen en/of aanhechtingsplaatsen op de darmwand, waardoor het aantal pathogene bacteriën zal afnemen en infecties worden voorkomen of verminderd. Dergelijke micro-organismen worden in het algemeen aangeduid met het begrip "probiotica".

Voor een optimale werking van deze micro-organismen dienen deze levend de darm te bereiken. Een verder gunstig effect van het toedienen van levende micro-organismen aan de darm is bijvoorbeeld dat zij de aldaar aanwezige oligosacchariden kunnen fermenteren, waarbij bijvoorbeeld vetzuren met korte keten kunnen worden gevormd.

Daarnaast is het om economische redenen gunstig wanneer zoveel mogelijk micro-organismen levend de darm bereiken. Met de gebruikelijke preparaten die probiotica bevatten is het percentage micro-organismen dat levend de darm bereikt vaak gering.

Het is daarom een doel van de onderhavige uitvinding een preparaat te verschaffen dat dergelijke probiotica bevat, waarbij een hoog percentage van de toegediende micro-organismen levend de darm bereikt.

Het is tevens een doel van de onderhavige uitvinding een preparaat te verschaffen dat kan worden gebruikt voor de behandeling van aandoeningen van de darm en/of dat kan worden gebruikt voor profylactische behandeling van de darm.

De onderhavige uitvinding betreft nu een preparaat met gezondheidbevorderende werking, in het bijzonder voor het voorkomen en/of behandelen van aandoe-

ningen van het spijsverteringskanaal, meer in het bijzonder van de darm, dat probiotica en onverteerbare oligosacchariden bevat.

Zonder aan enige theorie gebonden te willen zijn, wordt aangenomen dat de oligosacchariden een substraat voor de probiotica vormen, waardoor de kans dat deze micro-organismen levend de darm bereiken groter wordt, en waardoor de kans dat ze in combinatie met de in de darm aanwezige oligosacchariden hun gunstige werking kunnen uitoefenen toeneemt. De oligosacchariden zouden derhalve ook kunnen worden aangeduid als "prebiotica".

De probiotica en onverteerbare oligosacchariden zijn in het preparaat aanwezig in een verhouding van 1 tot 5 g oligosacchariden per 10^8 tot 10^{11} cellen van het probioticum.

De gebruikte oligosacchariden worden gekozen uit de zgn. "onverteerbare oligosacchariden", d.w.z. oligosacchariden die niet door het menselijk of dierlijk lichaam worden opgenomen. Deze oligosacchariden hebben in de regel een polymerisatiegraad van 2 tot 20. Dit houdt in dat de oligosacchariden bestaan uit 2 tot 20 monosaccharide-eenheden. Bij een polymerisatiegraad kleiner dan 2, dat wil zeggen bij het monosaccharide, is het preparaat niet werkzaam aangezien dergelijke monosacchariden door het menselijk of dierlijk lichaam worden opgenomen. Bij een polymerisatiegraad groter dan 20 verliezen de oligosacchariden hun gunstige werking.

Bij voorkeur hebben de oligosacchariden een polymerisatiegraad van 2 tot 10, met nog meer voorkeur van 2 tot 6, meer in het bijzonder 3 tot 10, met meer voorkeur 3 tot 6.

Wanneer in de onderhavige aanvrage wordt gesproken over oligosacchariden wordt hiermee zowel oligosacchariden met één bepaalde ketenlengte als mengsels van oligosacchariden met verschillende ketenlengten bedoeld. De voorkeur gaat echter uit naar een mengsel van oligosacchariden met verschillende ketenlengte.

Verder bestaan de oligosacchariden doorgaans niet geheel uit disacchariden. Het gehalte aan disacchariden is meestal minder dan 90%, soms minder dan 60%.

De gemiddelde polymerisatiegraad is in de regel meer dan 2,1, doorgaans meer dan 2,5.

De bij de uitvinding toegepaste oligosacchariden worden in de regel verder zodanig gekozen, dat zij voor ten minste 20% bruikbaar zijn als substraat voor de in het preparaat aanwezige probiotische micro-organismen, zoals bepaald volgens high

performance anion exchange chromatography.

Voorbeelden van geschikte oligosacchariden zijn transgalacto-oligosacchariden (TOS), fructo-oligosacchariden (FOS), en combinaties hiervan.

Het is bijzonder gunstig wanneer de oligosacchariden aan het preparaat worden toegevoegd in de vorm van een hydrolysaat van een of meer polysacchariden, bijvoorbeeld gekozen uit β-(arabino)galactanen, β-(arabino)xylanen, β-glucanen, β-glucomannanen, β-galactomannanen, α-arabanen, inuline, en combinaties hiervan. Een dergelijk hydrolysaat kan naast de oligosacchariden ook nog andere componenten bevatten zoals monosacchariden en sacchariden met een hogere polymerisatiegraad dan 20. Het hydrolysaat dient echter ten minste 50% onverteerbare oligosacchariden te bevatten, bij voorkeur ten minste 70%.

De polysacchariden die bij voorkeur gehydrolyseerd worden zijn β-(arabino)galactanen, β-mannanen, en xylanen.

Ook kan een hydrolyseproduct worden gebruikt van een of meer vezels die voor het merendeel uit de bovengenoemde oligosacchariden zijn opgebouwd, zoals vezels uit haver, tarwe, aardappel, suikerbiet, soyapolysacchariden en dergelijke.

De hydrolyse van de polysaccharide(n) en/of de vezel(s) kan op een op zichzelf bekende wijze worden uitgevoerd, bijvoorbeeld door de toepassing van geschikte enzymen.

De concentratie oligosacchariden in het preparaat is zodanig dat per dag 0,5 tot 20 gram kan worden toegediend. Indien dit gewenst is, kan deze toediening over de dag worden gespreid, zolang het totaal van de toegediende oligosacchariden binnen het hiervoor beschreven traject blijft. In het algemeen zal het preparaat 2 tot 4 keer per dag worden toegediend.

In het algemeen zullen de oligosacchariden 5 gew.% tot 50 gew.% van het totale preparaat uitmaken.

De voor de onderhavige uitvinding geschikte probiotica zijn algemeen bekend. Zij omvatten in het algemeen een of meer voor toepassing in voedingspreparaten geschikte bacteriestammen, zoals voor toepassing in voedingspreparaten geschikte melkzuurbacteriën, of een of meer voor toepassing in voedingspreparaten geschikte giststammen, of een combinatie hiervan. Deze probiotica zullen meestal de status GRAS ("Generally Recognized As Safe") hebben.

Geschikte bacteriestammen worden bijvoorbeeld gekozen uit die welke

worden beschreven in de Europese aanvrage 97202900.3 van aanvraagster. Andere mogelijke probiotica zijn de soorten der Pediococcen, Propionibacteriën of *Leuconostoc*. De voorkeur verdienen de geslachten *Lactobacillus*, *Bifidobacterium*, en combinaties hiervan.

Als de *Bifidobacterium* stam kan iedere stam worden gebruikt die geschikt is voor, en bij voorkeur ook goedgekeurd is voor, toediening aan mensen en dieren, zoals *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis* of *Bifidobacterium longum*, of een combinatie daarvan.

De *Lactobacillus* stam wordt bij voorkeur zodanig gekozen dat deze voornamelijk, bij voorkeur uitsluitend rechtsdraaiend (L+) lactaat produceert. Hiermee wordt bedoeld dat van het geproduceerde lactaat minder dan 5 %, bij voorkeur minder dan 2% linksdraaiend lactaat is. Het is natuurlijk mogelijk dat het micro-organisme naast lactaat andere metabolieten produceert, en de gunstige werking van het micro-organisme kan (tevens) op de vorming van deze verdere metabolieten berusten.

Voorbeelden hiervan zijn *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus (para)caseï, Lactobacillus fermentum, Lactobacillus plantarum en Lactobacillus rhamnosus*, en combinaties hiervan.

Als de bij de uitvinding toegepaste giststam kan iedere stam worden gebruikt die geschikt is voor, en bij voorkeur ook goedgekeurd is voor, toediening aan mensen en dieren, zoals van het geslacht *Saccharomyces*. Voorbeelden van geschikte *Saccharomyces*-soorten zijn *Saccharomyces cerevisiae* en *Saccharomyces boulardii*.

De gist in het preparaat kan levend of dood zijn. Zowel dode als levende gist bevat een hoog gehalte aan mannoproteïnen, die in sterke mate de hechting van bacteriën aan de darmwand kunnen voorkomen. Toediening van dode gist biedt voordelen bij mensen die lijden aan inflammatoire darmziekten.

Wanneer de gist in dode vorm wordt gebruikt, dient ten minste een ander levend micro-organisme te worden toegepast. Dit levend organisme kan weer worden gekozen uit zowel de stammen *Lactobacillus*, *Bifidobacterium*, Pediococcen, Propionibacteriën als *Leuconostoc*, maar kan ook levend *Saccharomyces* zijn.

Er kan zowel een enkel micro-organisme als een mengsel van micro-organismen worden gebruikt.

De totale concentratie van de probiotica is 10^6 tot 10^{12}, bij voorkeur 10^9 tot 10^{10} levende cellen per gram totaal produkt. Wanneer een combinatie van micro-

organismen wordt gebruikt, geldt dat de minimale concentratie van elk van de micro-organismen zodanig moet zijn dat nog gesproken kan worden van levende organismen, dat wil zeggen minimaal ongeveer 10 per gram produkt. De totale concentratie micro-organismen dient steeds binnen het hiervoor vermelde traject van 10^6 tot 10^{12} levende cellen per gram totaal produkt te liggen.

Wanneer eveneens dood *Saccharomyces cerevisiae* wordt toegepast, wordt dit in een hoeveelheid van 0,5 tot 5 g per dag toegediend.

Een bijzonder geschikte combinatie van oligosaccharide en probioticum blijkt de combinatie van *Lactobacillus rhamnosus* met transgalacto-oligosaccharide of hydrolysaten van (aardappel)galactaan te zijn.

De geschiktheid van een bepaald oligosaccharide voor een bepaald probioticum kan worden bepaald door het vermogen te meten van het betreffende micro-organisme om dat oligosaccharide of die oligosaccharidefractie te fermenteren. Een specifieke methode hiervoor is hierna bij de voorbeelden weergegeven.

De toedieningsvormen van de preparaten volgens de uitvinding zijn in de regel analoog aan die welke zijn beschreven in de Europese aanvrage 97202900.3 van aanvraagster, waarvan de inhoud hierin door verwijzing is opgenomen. Hierbij dient opgemerkt te worden dat volgens de onderhavige uitvinding ook slechts één probiotisch micro-organisme (waaronder een gist) aanwezig kan zijn, en dat naast de een of meer probiotische micro-organismen tevens een of meer oligosacchariden worden opgenomen, in de hierboven vermelde hoeveelheden.

De preparaten volgens de onderhavige uitvinding kunnen onder andere worden toegediend in de vorm van een voedingssupplement, een totale voeding en een klinische voeding. Voor de specifieke additieven die aan dergelijke voedingen worden toegevoegd, de bereiding en de toepassingen van dergelijke voedingen wordt eveneens verwezen naar de Europese aanvrage 97202900.3.

De probiotica worden bij voorkeur in ge(vries)droogde vorm aan het preparaat toegevoegd. Het is ook mogelijk vloeibare preparaten te vervaardigen, maar deze dienen gekoeld bewaard te worden. Verder kunnen een of meer van de micro-organismen in ingekapselde vorm worden gebruikt, bijvoorbeeld voor het verbeteren van de houdbaarheid.

Wanneer het preparaat volgens de uitvinding wordt gebruikt als voedingssupplement, kan het als zodanig kan worden toegediend, kan het worden gemengd

met een geschikte drinkbare vloeistof zoals water, yoghurt, melk of vruchtesap, of kan het worden gemengd met vast of vloeibaar voedsel. Hierbij kan het voedingssupplement in de vorm zijn van tabletten, capsules, poeders, sachets, pastilles, snoepjes, repen en overeenkomstige toedieningsvormen, doorgaans in de vorm van een eenheidsdosering.

Een supplement volgens de uitvinding kan bijvoorbeeld de volgende samenstelling hebben:

- probiotica: 10 - 40 gew. %
- oligosacchariden: 40 - 80 gew. %
- verdere toevoegsels: 0 - 40 gew. %,

tot een totaal van 100 gew.%

Het preparaat kan ook in de vorm zijn van een voedingspreparaat dat geschikt is voor directe consumptie, zoals een totale of klinische voeding. Dit kan zowel een orale voeding als een voeding voor toediening via een buis of catheter zijn.

Dergelijke voedingen kunnen in vaste vorm of vloeibare/drinkbare vorm zijn, en kunnen alle gebruikelijke toevoegsels voor (totale en/of klinische) voedingen bevatten, waaronder eiwitten, vitaminen, mineralen, spore-elementen en dergelijke.

Een totale voeding volgens de uitvinding kan bijvoorbeeld de volgende samenstelling hebben:

- probiotica: 0,1 - 10 gew. %
- oligosacchariden: 1 - 20 gew. %
- verdere toevoegsels: 75 - 95 gew. %,

tot een totaal van 100 gew.%.

Volgens een bijzondere uitvoeringsvorm is het preparaat volgens de uitvinding in de vorm van een (supplement voor een) zuigelingenvoeding of een voedingssupplement voor zuigelingen.

De hiervoor beschreven preparaten kunnen worden gebruikt voor dezelfde toepassingen als omschreven in de Europese aanvrage 97202900.3 van aanvraagster, in het bijzonder bij de behandeling van aandoeningen van de darm zoals diarree zoals kan optreden bij reizen, na behandeling met antibiotica of die het gevolg is van voedselvergiftiging. Een andere toepassing is bij de behandeling van inflammatoire darmziekten (IBD), zoals colitis ulcerosa en de ziekte van Crohn. De preparaten volgens de uitvinding zijn eveneens geschikt voor patiënten met een voedselallergie,

zoals een allergie voor koemelk of voor gluten.

Tevens kunnen de probiotica en onverteerbare oligosacchariden gebruikt worden in zuigelingenvoeding ter voorkoming of behandeling van darmproblemen.

De uitvinding wordt nu toegelicht aan de hand van de volgende niet-beperkende voorbeelden.

Voorbeelden

Om geschikte combinaties van oligosacchariden en probiotica te bepalen, werden de hierna weergegeven micro-organismen getest op hun vermogen structureel verschillende oligosaccharidefracties te fermenteren. Stammen voorgekweekt in vloeibaar medium op basis van thioglycolaat (Oxoid, Unipath LTD, Basingstoke, Hampshire, UK) werden aan subkweek onderworpen in thioglycolaat waaraan 0.5% (w/v) oligosacchariden waren toegevoegd. Het suikervrije thioglycolaat-medium alsmede de oligosaccharide-oplossingen werden afzonderlijk 15 minuten bij 121 °C gesteriliseerd.

Na anaërobe incubatie gedurende 48 uur bij 37 °C werd de pH gemeten met behulp van een micro-pH meter (Sentron, Roden, Nederland). De veranderingen in het gehalte resterende oligosacchariden, en de vorming van reactieprodukten werden gemeten met HPAEC (High Performance Anion Exchange Chromatography). Voor de HPAEC analyse werden de kweken gecentrifugeerd, werd het supernatans 10 keer verdund met H_2O en 5 minuten gekookt om de enzymatische activiteit te stoppen. Voor en na fermentatie werd de zuiverheid van de stammen gecontroleerd.

Het HPAEC systeem bestond uit een Dionex Bio-LC GPM-II quartenaire gradiëntmodule (Dionex Corporation, Sunnyval, CA, USA) uitgerust met een Dionex Carbopac PA-100 kolom (4 * 250 mm) in combinatie met een Carbopac PA-100 guard kolom (3 * 25 mm). De monsters (20 µl) werden geïnjecteerd met een Spectra Physics SP8880 autosampler. De oligomeren werden geanalyseerd met een gradiënt van natriumacetaat in 100 $mmol.l^{-1}$ NaOH.

De resultaten van deze experimenten zijn hierna in de tabel weergegeven voor een aantal combinaties van probiotica en substraten, waarbij:

++ staat voor volledige fermentatie

+ staat voor gedeeltelijke fermentatie

- staat voor geen of zeer beperkte fermentatie

Bacteriën	Substraat			
	Hydrolysaten van:			
	Arabino-galactanen	Arabanen	Arabino-xylanen	Fructanen
Bi. Breve	++	+	-	++
Bi. Longum	++	++	+	++
Bi. adolescentis	++	+	++	++
L. acidophilus	++	-	-	+
L. fermentum	++	-	-	+
K. pneumoniae	++	-	-	+
C. perfingens	-	-	-	++

Hierna worden enkele voorbeelden weergegeven van preparaten volgens de onderhavige uitvinding.

Voorbeeld I: Supplement

Een suspensie van *Lactobacillus rhamnosus* ATCC 7469 (Lb) werd gevriesdroogd, waarbij een poeder werd verkregen met ten minste 10^9 levensvatbare cellen per gram poeder. Transgalacto-oligosacchariden (TOS), verkregen uit lactose (Borculo Whey Products), werden opgelost in water van 40°C tot een vaste stoffengehalte van 25%, en deze oplossing werd gesproeidroogd. Beide poeders werden gemengd in een verhouding TOS/Lb = 4/1 tot een homogeen product werd verkregen. Sachets werden gevuld met 2-5 g van dit mengsel, afhankelijk van het doseringsregime (5 g voor één sachet per dag; 3 g voor 2 sachets per dag). De inhoud van een sachet kan bijvoorbeeld gemengd met een glas sinaasappelsap of melk worden ingenomen.

Voorbeeld II: Synbiotische reep

Een reep van 23 g werd bereid uit 4,0 g havervlokken, 4,0 g tarwevlokken, 3,0 g gepofte rijst, 1,0 g opgebroken hazelnoten, 0,25 g honing, 3,0 g rozijnen, 1,5 g maltodextrine, 1,0 g gevriesdroogde *Lb rhamnosus*, 0,5 g bakkersgist (*Saccharomyces*

cerevisiae; Gist Brocades), en 5,0 g transgalacto-oligosacchariden.

Voorbeeld III: Werkwijze voor het hydrolyseren van plantaardige vezels

Een 20% suspensie in water van vezels, bijvoorbeeld uit tarwe, aardappel, haver, soya-polysacchariden, johannesbroodgom, of suikerbiet werd bereid. Deze vezelbronnen zijn commercieel verkrijgbaar. De suspensie werd verhit tot een temperatuur tussen 20 en 50°C (bij voorkeur 35-45°C) waarna enzymen werden toegevoegd in een hoeveelheid van 1 deel enzym per 5-500 delen (w/w) substraat. De keuze van het soort enzym is afhankelijk van het soort polysaccharide. Voorbeelden van geschikte enzymen zijn Novoferm, Pectinex Ultra s.p.-L, Pentopan en Ultra.s.p. (NOVO).

Na 0,5-4 uur werd de reactie beëindigd door verhitten, waarna de aldus verkregen oplossing kan worden gebruikt als de oligosaccharide-fractie in de preparaten volgens de uitvinding, eventueel na verdere filtratie/zuivering of na concentreren.

Voorbeeld IV: Synbiotisch mengsel voor mengen met een complete enterale klinische voeding.

Een moedercharge van een poedermengsel werd bereid volgens de werkwijze van voorbeeld I. Het poeder bestond uit 20% gehydrolyseerde, tarwe-arabinoxylanen, 20% gehydrolyseerde aardappel-arabinogalactanen, 20% gehydrolyseerde johannes-broodgom, 20% gehydrolyseerde suikerbietvezel (arabanen), 15% gehydrolyseerde haver vezels (glucanen) en 5% *Bifidobacterium longum*. 5 g van het poedermengsel wordt in een sachet gebracht. De inhoud van dit sachet kan maximaal 30 min. voor gebruik aan een standaard enterale klinische voeding worden toegevoegd.

Voorbeeld V: Synbiotisch poedermengsel voor het versterken van zuigelingen-voeding.

Een synbiotisch mengsel werd bereid volgens de werkwijze van Voorbeeld I. De samenstelling omvat 10% bakkersgist (Gist Brocades), 40% mannoproteinen, verkregen uit gist, 25% inuline en 25% raffinose.

Voorbeeld VI: Snoep dat een synbiotisch mengsel omvat.

Een snoepje van 2 g werd bereid, uitgaande van 58% verteerbare koolhydraten (glucosesiroop), 35% TOS, 4% *Lactobacillus rhamnosus* ATCC 7469, en 2% smaak- en kleurstoffen.

Conclusies

1. Preparaat met gezondheidbevorderende werking, in het bijzonder voor het voorkomen en/of behandelen van aandoeningen van het spijsverteringskanaal, dat een of meer probiotica en een of meer onverteerbare oligosacchariden bevat.

2. Preparaat volgens conclusie 1, waarbij de oligosacchariden een polymerisatiegraad van 2 tot 20, bij voorkeur van 2 tot 10, met nog meer voorkeur van 2 tot 6 hebben.

3. Preparaat volgens conclusie 1 of 2, waarbij de oligosacchariden zijn verkregen uit de hydrolyse van een of meer polysacchariden, gekozen uit gekozen uit β-(arabino)galactanen, β-(arabino)xylanen, β-glucanen, β-glucomannanen, β-galactomannanen, α-arabanen, inuline, en combinaties hiervan.

4. Preparaat volgens conclusie 3, waarbij de polysacchariden worden gekozen uit β-(arabino)galactanen, β-mannanen, en xylanen, en combinaties hiervan.

5. Preparaat volgens conclusie 1 of 2, waarbij de oligosacchariden afkomstig zijn uit de hydrolyse van een of meer vezels, zoals vezels afkomstig uit haver, tarwe, aardappel, suikerbiet, soyapolysacchariden en dergelijke.

6. Preparaat volgens een van de voorgaande conclusies, waarbij de probiotica ten minste een bacteriestam of ten minste een giststam, of een combinatie van ten minste een bacteriestam en ten minste een giststam omvatten.

7. Preparaat volgens een van de voorgaande conclusies, waarbij ten minste een van de bacteriestammen wordt gekozen uit een of meer stammen van een *Lactobacillus*- of een *Bifidobacterium*-soort en de giststam een stam van een *Saccharomyces*-soort is.

8. Preparaat volgens een van de voorgaande conclusies, dat voorts dode gistcellen omvat.

9. Preparaat volgens een der voorafgaande conclusies, waarbij verhouding tussen de een of meer probiotica en de een of meer onverteerbare oligosacchariden in het bereik van 1 tot 5 g oligosacchariden per 10^8 tot 10^{11} cellen van het probioticum ligt.

10. Preparaat volgens een van de voorgaande conclusies, dat de probiotica in een concentratie van 10^7 tot 10^{11} levende cellen per gram totaal product bevat.

Uittreksel

De onderhavige uitvinding heeft betrekking op een preparaat met gezondheid-bevorderende werking, in het bijzonder voor het voorkomen en/of behandelen van aandoeningen van het spijsverteringskanaal, dat een of meer probiotica en een of meer onverteerbare oligosacchariden bevat. De probiotica worden bij voorkeur gekozen uit bacteriestammen zoals een stam van een *Lactobacillus*- of een *Bifido-bacterium*-soort en uit giststammen zoals een stam van een *Saccharomyces*-soort.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 35/74, 35/72, A23L 1/03, 1/308

A1

(11) International Publication Number: **WO 00/33854**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/NL99/00755

(22) International Filing Date: 9 December 1999 (09.12.99)

(30) Priority Data:
1010770 9 December 1998 (09.12.98) NL

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
In English translation (filed in Dutch).

(54) Title: PREPARATION THAT CONTAINS OLIGOSACCHARIDES AND PROBIOTICS

(57) Abstract

The present invention relates to a preparation having a health–promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, which contains one or more probiotics and one or more non–digestible oligosaccharides. The probiotics are preferably chosen from bacterial strains such as a strain of a *Lactobacillus* or a *Bifidobacterium* species and from yeast strains such as a strain of a *Saccharomyces* species.

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Preparation that contains oligosaccharides and probiotics

The present invention relates to a preparation which has a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, more particularly of the intestines.

The application relates in particular to a preparation of this type which contains probiotics and non-digestible oligosaccharides.

It is known that certain microorganisms have both a prophylactic and a therapeutic effect on intestinal diseases, such as intestinal infections. When these microorganisms are administered to humans or animals they will compete with pathogenic bacteria for nutrients and/or adhesion sites on the intestinal wall, as a result of which the number of pathogenic bacteria will decrease and infections are prevented or reduced. Such microorganisms are generally designated by the term "probiotics".

If these microorganisms are to have an optimum action they must reach the intestines alive. A further beneficial effect of the administration of live microorganisms to the intestines is, for example, that they are able to ferment the oligosaccharides present in the intestines, whereby, for example, fatty acids with short chains are formed.

In addition it is advantageous on economic grounds if as many microorganisms as possible reach the intestines alive. With the customary preparations which contain probiotics the percentage of microorganisms that reaches the intestines alive is frequently low.

It is therefore an object of the present invention to provide a preparation that contains such probiotics with which a high percentage of the microorganisms administered reaches the intestines alive.

It is also an object of the present invention to provide a preparation that can be used for the treatment of disorders of the intestines and/or that can be used for prophylactic treatment of the intestines.

The present invention now relates to a preparation having a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, more particularly of the intestines, which contains probiotics and non-digestible oligosaccharides.

Without wishing to be tied to any theory, it is assumed that the oligosaccharides form a substrate for the probiotics, as a result of which the likelihood that said

microorganisms reach the intestines alive increases and as a result of which the likelihood that they are able, in combination with the oligosaccharides present in the intestines, to exert their beneficial action increases. The oligosaccharides could therefore also be designated as "prebiotics".

The probiotics and non-digestible oligosaccharides are present in the preparation in a ratio of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

The oligosaccharides used are chosen from the so-called "non-digestible oligosaccharides", that is to say oligosaccharides which are not absorbed by the human or animal body. These oligosaccharides as a rule have a degree of polymerisation of 2 to 20. This implies that the oligosaccharides consist of 2 to 20 monosaccharide units. In the case of a degree of polymerisation of less than 2, that is to say in the case of the monosaccharide, the preparation is not effective since such monosaccharides are absorbed by the human or animal body. At a degree of polymerisation of greater than 20 the oligosaccharides lose their beneficial action.

Preferably the oligosaccharides have a degree of polymerisation of 2 to 10, more preferentially of 2 to 6, more particularly 3 to 10 and more preferentially 3 to 6.

Where reference is made in the present application to oligosaccharides this term is used to refer both to oligosaccharides having one specific chain length and to mixtures of oligosaccharides having different chain lengths. However, a mixture of oligosaccharides having different chain lengths is preferred.

Furthermore, the oligosaccharides usually do not consist entirely of disaccharides. The disaccharide content is usually less than 90 % and sometimes less than 60 %.

The average degree of polymerisation is as a rule more than 2.1, usually more than 2.5.

The oligosaccharides used in the invention are, as a rule, furthermore so chosen that they are at least 20 % usable as substrate for the probiotic microorganisms present in the preparation, as determined by high performance anion exchange chromatography.

Examples of suitable oligosaccharides are transgalacto-oligosaccharides (TOS), fructo-oligosaccharides (FOS) and combinations thereof.

It is particularly advantageous if the oligosaccharides are added to the preparation in the form of a hydrolysis product of one or more polysaccharides, for example chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof. In addition to the oligosaccharides, such a

hydrolysis product can also contains yet further components, such as monosaccharides and saccharides having a degree of polymerisation higher than 20. However, the hydrolysis product must contain at least 50 % non-digestible oligosaccharides, preferably at least 70 %.

The polysaccharides which are preferably hydrolysed are β-(arabino)galactans, β-mannans, and xylans.

It is also possible to use a hydrolysis product of one or more fibres which are mainly made up of the abovementioned oligosaccharides, such as fibres from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

The hydrolysis of the polysaccharide(s) and/or the fibre(s) can be carried out in a manner known per se, for example by the use of suitable enzymes.

The concentration of oligosaccharides in the preparation is such that 0.5 to 20 gram per day can be administered. If desired, this administration can be spread over the day, as long as the total quantity of the oligosaccharides administered remains in the range described above. In general the preparation will be administered 2 to 4 times per day.

In general the oligosaccharides will make up 5 wt.% to 50 wt.% of the total preparation.

The probiotics suitable for the present invention are generally known. They comprise, in general, one or more bacterial strains suitable for use in food preparations, such as lactic acid bacteria suitable for use in food preparations, or one or more yeast strains suitable for use in food preparations, or a combination thereof. These probiotics will usually have GRAS ("Generally Recognised As Safe") status.

Suitable bacterial strains are, for example, chosen from those which are described in European Application 97202900.3 in the name of the Applicant. Other possible probiotics are the Pediococci, Propionibacteria or *Leuconostoc* species. The *Lactobacillus*, and *Bifidobacterium* genera and combinations thereof are to be preferred.

The *Bifidobacterium* strain used can be any strain which is suitable for, and preferably is also approved for, administration to humans and animals, such as *Bifidobacterium, bifidum, Bifidobacterium breve, Bifidobacterium lactis* or *Bifidobacterium longum*, or a combination thereof.

The *Lactobacillus* strain is preferably so chosen that this produces mainly, preferably exclusively, dextrorotatary (L+) lactate. What is meant by this is that the lactate produced is less than 5 %, preferably less than 2 %, laevorotatory lactate. It is, of course, possible

that the microorganism produces other metabolites in addition to lactate and the beneficial action of the microorganism can (also) be based on the formation of these further metabolites.

Examples of these are *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus (para)casei, Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus rhamnosus*, and combinations thereof.

The yeast strain used in the invention can be any strain which is suitable for, and preferably is also approved for, administration to humans and animals, such as of the genus *Saccharomyces*. Examples of suitable *Saccharomyces* species are *Saccharomyces cerevisiae* and *Saccharomyces boulardii*.

The yeast in the preparation can be alive or dead. Both dead and live yeast contains a high content of mannoproteins, which are able to prevent the adhesion of bacteria to the intestinal wall to a large extent. Administration of dead yeast offers advantages in the case of people who are suffering from inflammatory intestinal diseases.

If the yeast is used in dead form, at least one other live microorganism must be used. Said live organism can once again be chosen from both the *Lactobacillus, Bifidobacterium*, Pediococci, Propionibacteria and *Leuconostoc* strains, but can also be live *Saccharomyces*.

It is possible to use both a single microorganism and a mixture of microorganisms.

The total concentration of the probiotics is 10^6 to 10^{12}, preferably 10^9 to 10^{10}, live cells per gram of total product. If a combination of microorganisms is used, the minimum concentration of each of the microorganisms must be such that there can still be said to be live organisms, that is to say at least approximately 10 per gram of product. The total concentration of microorganisms must always be within the above specified range of 10^6 to 10^{12} live cells per gram of total product.

If dead *Saccharomyces cerevisae* is also used, this is administered in a quantity of 0.5 to 5 g per day.

The combination of *Lactobacillus rhamnosus* with transgalacto-oligosaccharide or hydrolysis products of (potato) galactan is found to be a particularly suitable combination of oligosaccharide and probiotic.

The suitability of a specific oligosaccharide for a specific probiotic can be determined by determining the capacity of the microorganism concerned to ferment said oligosaccharide or said oligosaccharide fraction. A specific method for this is given below

with the examples.

The administration forms of the preparations according to the invention are as a rule analogous to those which have been described in European Application 97202900.3 in the name of the Applicant, the contents of which are incorporated herein by reference. In this context it must be pointed out that according to the present invention also only one probiotic microorganism (including a yeast) can be present and that in addition to the one or more probiotic microorganisms one or more oligosaccharides can also be incorporated, in the quantities specified above.

The preparations according to the present invention can, among others, be administered in the form of a nutritional supplement, total nutrition and clinical nutrition. Reference is likewise made to European Application 97202900.3 for the specific additives which are added to such foods and the preparation and applications of such foods.

The probiotics are preferably added to the preparation in (freeze-)dried form. It is also possible to produce liquid preparations, but these must be stored cool. Furthermore, one or more of the microorganisms can be used in encapsulated form, for example in order to improve the shelf life.

If the preparation according to the invention is used as a nutritional supplement, it can be administered as such, can be mixed with a suitable drinkable liquid, such as water, yoghurt, milk or fruit juice, or can be mixed with solid or liquid food. In this context the nutritional supplement can be in the form of tablets, capsules, powders, sachets, pastilles, sweets, bars and corresponding administration forms, usually in the form of a unit dose.

A supplement according to the invention can, for example, have the following composition:

- probiotics: 10 - 40 wt.%
- oligosaccharides: 40 - 80 wt.%
- further additives: 0 - 40 wt.%,

to a total of 100 wt.%.

The preparation can also be in the form of a food preparation that is suitable for direct consumption, such as total or clinical nutrition. This can be either oral nutrition or nutrition for administration via a tube or catheter.

Such foods can be in solid form or liquid/drinkable form and can contain all customary additives for (total and/or clinical) nutrition, including proteins, vitamins, minerals, trace elements and the like.

A total nutrition according to the invention can, for example, have the following composition:

- probiotics: 0.1 - 10 wt.%
- oligosaccharides: 1 - 20 wt.%
- further additives: 75 - 95 wt.%

to a total of 100 wt.%.

According to a particular embodiment, the preparation according to the invention is in the form of a (supplement for a) baby food or a nutritional supplement for babies.

The preparations described above can be used for the same applications as those described in European Application 97202900.3 in the name of the Applicant, in particular in the treatment of disorders of the intestines, such as diarrhoea, such as can arise when travelling or after treatment with antibiotics, or which results from food poisoning. Another application is in the treatment of inflammatory bowel diseases (IBD), such as colitis ulcerosa and Crohn's disease. The preparations according to the invention are also suitable for patients who have a food allergy, such as an allergy to cow's milk or to gluten.

The probiotics and non-digestible oligosaccharides can also be used in baby food to prevent or treat intestinal problems.

The invention will now be explained with the aid of the following non-limiting examples.

Examples

To determine suitable combinations of oligosaccharides and probiotics the microorganisms listed below were tested to determine their capacity to ferment structurally different oligosaccharide fractions. Strains pre-cultured in liquid medium based on thioglycolate (Oxoid, Unipath Ltd, Basingstoke, Hampshire, UK) were subjected to sub-culture in thioglycolate to which 0.5 % (m/V) oligosaccharides were added. The sugar-free thioglycolate medium and the oligosaccharide solutions were sterilised separately for 15 minutes at 121 °C.

Following anaerobic incubation for 48 hours at 37 °C the pH was measured with the aid of a micro-pH meter (Sentron, Roden, The Netherlands). The changes in the residual oligosaccharide content and the formation of reaction products were determined using HPAEC (high performance anionic exchange chromatography). For HPAEC analysis the cultures were centrifuged and the supernatant liquor was diluted 10-fold with H_2O and

boiled for 5 minutes to stop the enzymatic activity. The purity of the strains was checked before and after fermentation.

The HPAEC system consisted of a Dionex Bio-LC GPM-II quaternary gradient module (Dionex Corporation, Sunnyval, CA, USA) equipped with a Dionex Carbopac PA-100 column (4 * 250 mm) in combination with a Carbopac PA-100 guard column (3 * 25 mm). The samples (20 µl) were injected using a Spectra Physics SP8880 autosampler. The oligomers were analysed using a gradient of sodium acetate in 100 mmol.l^{-1} NaOH.

The results of these experiments are given below in the table for a number of combinations of probiotics and substrates, where:

++ indicates complete fermentation

\+ indicates partial fermentation

\- indicates no or very limited fermentation

Bacteria	Substrate			
	Hydrolysis product of:			
	Arabino-galactans	Arabans	Arabinoxylans	Fructans
Bi. Breve	++	+	-	++
Bi. Longum	++	++	+	++
Bi. adolescentis	++	+	++	++
L. acidophilus	++	-	-	+
L. fermentum	++	-	-	+
K. pnuemoniae	++	-	-	+
C. perfingens	-	-	-	++

A few examples of preparations according to the present invention are given below.

Example I: Supplement

A suspension of *Lactobacillus rhamnosus* ATCC 7469 (Lb) was freeze-dried, a powder being obtained which contained at least 10^9 viable cells per gram powder. Transgalacto-oligosaccharides (TOS), obtained from lactose (Borculo Whey Products), were dissolved in water at 40 °C to a solids content of 25 % and this solution was spray-dried. The two powders were mixed in a TOS/Lb ratio of 4/1 until a homogeneous product was obtained. Sachets were filled with 2 - 5 g of this mixture, depending on the dosage

regime (5 g for one sachet per day; 3 g for two sachets per day). The contents of one sachet can, for example, be taken mixed in a glass of orange juice or milk.

Example II: Synbiotic bar

A 23 g bar was prepared from 4.0 g oat flakes, 4.0 g wheat flakes, 3.0 g puffed rice, 1.0 g crushed hazelnuts, 0.25 g honey, 3.0 g raisins, 1.5 g maltodextrin, 1.0 g freeze-dried *Lb rhamnosus*, 0.5 g baker's yeast (*Saccharomyces cerevisiae*; Gist Brocades) and 5.0 g transgalacto-oligosaccharides.

Example III: Method for hydrolysing vegetable fibres

A 20 % suspension of fibres, for example from wheat, potatoes, oats, soya polysaccharides, carob gum or sugar beet, in water was prepared. These sources of fibre are commercially available. The suspension was heated to a temperature of between 20 and 50 °C (preferably 35 - 45 °C), after which enzymes were added in a quantity of one part enzyme per 5 - 500 parts (m/m) substrate. The choice of the type of enzyme is dependent on the type of polysaccharide. Examples of suitable enzymes are Novoferm Pectinex Ultra s.p.-L, Pentopan and Ultra.s.p. (NOVO).

After 0.5 - 4 hours the reaction was terminated by heating, after which the solution thus obtained can be used as the oligosaccharide fraction in the preparations according to the invention, optionally after further filtration/purification or after concentrating.

Example IV: Synbiotic mixture for mixing with a complete enteral clinical nutrition

A mother batch of a powder mixture was prepared in accordance with the method of Example I. The powder consisted of 20 % hydrolysed wheat arabinoxylans, 20 % hydrolysed potato arabinogalactans, 20 % hydrolysed carob gum, 20 % hydrolysed sugar beet fibre (arabans), 15 % hydrolysed oat fibres (glucans) and 5 % *Bifidobacterium longum*. 5 g of the powder mixture is placed in a sachet. The contents of this sachet can be added to a standard enteral clinical nutrition a maximum of 30 min before use.

Example V: Synbiotic powder mixture for fortifying baby food

A synbiotic mixture was prepared in accordance with the method of Example I. The composition contains 10 % baker's yeast (Gist Brocades), 40 % mannoproteins, obtained from yeast, 25 % inulin and 25 % raffinose.

Example VI: Sweet that contains a synbiotic mixture

A 2 g sweet was prepared starting from 58 % digestible carbohydrates (glucose syrup), 35 % TOS, 4 % *Lactobacillus rhamnosus* ATCC 7469, and 2 % flavourings and colourants.

CLAIMS

1. Preparation having a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, which contains one or more probiotics and one or more non-digestible oligosaccharides.

2. Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerisation of 2 to 20, preferably of 2 to 10, more preferentially of 2 to 6.

3. Preparation according to Claim 1 or 2, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.

4. Preparation according to Claim 3, wherein the polysaccharides are chosen from β-(arabino)galactans, β-mannans, xylans and combinations thereof.

5. Preparation according to Claim 1 or 2, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

6. Preparation according to one of the preceding claims, wherein the probiotics comprise at least one bacterial strain or at least one yeast strain, or a combination of at least one bacterial strain and at least one yeast strain.

7. Preparation according to one of the preceding claims, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lactobacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a *Saccharomyces* species.

8. Preparation according to one of the preceding claims which also contains dead yeast cells.

9. Preparation according to one of the preceding claims, wherein the ratio between the

one or more probiotics and the one or more non-digestible oligosaccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

10. Preparation according to one of the preceding claims which contains the probiotics in a concentration of 10^7 to 10^{11} live cells per gram of total product.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 99/00755

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/74 A61K35/72 A23L1/03 A23L1/308

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 856 259 A (SITIA YOMO SPA) 5 August 1998 (1998-08-05) column 1, line 14-44 column 5, line 32 -column 6, line 12 claims	1-3,9,10
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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21 March 2000	31/03/2000

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European Patent Office
Office européen des brevets

(11) EP 0 806 144 A2

(12) EUROPÄISCHE PATENTANMELDUNG

(43) Veröffentlichungstag:
12.11.1997 Patentblatt 1997/46

(51) Int Cl.[6]: **A21D 8/04**

(21) Anmeldenummer: **97810293.7**

(22) Anmeldetag: **09.05.1997**

(84) Benannte Vertragsstaaten:
AT BE CH DE DK ES FI FR GB IE IT LI LU NL PT SE

(30) Priorität: **11.05.1996 DE 19619187**

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(54) Herstellung eines flüssigen bzw. pastösen biologischen Back-mittels für Brot mit Hilfe von Milchsäurebakterien sowie danach hergestelltes biologisches Backmittel

(57) Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung eines flüssigen oder pastösen biologischen Backmittels mit lebenden Milchsäurebakterienarten. Sie wird dadurch gekennzeichnet, dass das gesamte Nährmedium als Backmittel dient. Zur Herstellung des Nährmediums werden Getreiderohstoffe gemischt und mindestens eine Amylase und mindestens eine Amyloglucosidase zugegeben, wobei das Medium zuerst 20 Minuten auf 75°C und dann auf 55°C abgekühlt und nach Zugabe von mindestens 2 Proteasen 2 Stunden bei dieser Temperatur gehalten wird, woran sich dann die Sterilisation des Nährmediums anschliesst. Das Nährmedium wird danach mit den in der Erfindung genannten Milchsäurebakterienarten beimpft. Dem erfindungsgemässen Backmittel werden noch Ascorbinsäure, α - Amylasen und Malzmehl beigegeben.

EP 0 806 144 A2

Printed by Jouve, 75001 PARIS (FR)

Beschreibung

Die Erfindung betrifft die Herstellung eines flüssigen oder pastösen biologischen Backmittels mit Hilfe von Milchsäurebakterien, dem, je nach Anwendung und Brotrezeptur noch Enzyme, Malzmehl und Ascorbinsäure oder ascorbinsäurehaltige Fruchtpulver zugegeben werden können, sowie ein danach hergestelltes biologisches Backmittel.

Weizen- und Roggenbrote sowie Hefegebäcke werden heute unter Einsatz von verschiedenen Zusatzstoffen hergestellt. In den klassischen Verfahren werden Vorteige zur Verbesserung des Geschmackes und der Teiglockerung verwendet. Als Teigsäuerungsmittel und Geschmacksverbesserer sind gefriergetrocknete Lactobazillen, gefriergetrockneter Sauerteig, inaktive Gärflüssigkeit von Lactobazillen und chemisch hergestellte organische Säuren im Einsatz. Beim Gefriertrocknen gehen wesentliche flüchtige Aromakomponenten verloren. Alle eingesetzten Mikroorganismen werden unter Zusatz von chemisch hergestellten Nähr- und Wuchsstoffen gezüchtet. Getrocknete Mikroorganismen müssen erst revitalisiert werden. Durch die zum Teil fehlenden Enzyme zum Abbau von Getreideinhaltsstoffen kommt es zum verzögerten Wachstum (Lag-Phase). Mit Ausnahme von gefriergetrocknetem Sauerteig fehlt allen eingesetzten Zusatzstoffen der typische Brotgeschmack.Getrockneter Sauerteig muss bis zu 20% (bezogen auf den Mehlanteil) dem Teig zugesetzt werden.

Aufgabe der vorliegenden Erfindung ist es, ein biologisches Backmittel herzustellen, welches gegenüber den bekannten Backmitteln den Vorteil aufweist, dass es keine chemischen Zusätze enthält, dass es ohne Zugabe von Microorganismen, die unter Zusatz von chemisch hergestellten Nähr- und Wuchsstoffen gezüchtet wurden, auskommt, dass diese Microorganismen nicht revitalisiert werden müssen, dass kein verzögertes Wachstum auftritt, dass der typische Brotgeschmack erzielt wird, und dass eine wesentlich geringere Dosierung der Zugabemenge an getrocknetem Sauerteig erforderlich ist.

Die Lösung der vorstehend geschilderten Aufgaben wird durch die Merkmale des kennzeichnenden Teils des Anspruchs 1 ermöglicht.

Weitere Ausführungsformen des erfindungsgemässen Verfahrens sind in den Unteransprüchen beschrieben.

Das erfindungsgemässe biologische Backmittel in flüssiger oder pastöser Form vermeidet die Nachteile der z.Z. verwendeten Zusatzstoffe. Die Milchsäurebakterien-Arten werden auf Getreiderohstoffen, vorzugsweise aus biologisch-kontroliertem Anbau, gezüchtet. Das Medium enthält keine chemischen Zusätze. Während der Fermentation entstehen die für die Teigreifung typischen Aromastoffe. Da kein Konzentrierungsschritt stattfindet, gelangen alle Aromastoffe in den Teig und damit in die Brote. Die lebenden Milchsäurebakterien haben bereits die zum Abbau der Getreiderohstoffe nötigen Enzymsysteme aktiviert und können ohne Verzögerung ihren Stoffwechsel im Teig weiterführen. Je nach Teigart und Teigführung wird durch die Aromabildung und Teiglockerung in kurzer Zeit deutlich verbessert. Die Dosierung kann je nach Teigart 1% - 10%, vorzugsweise 2% - 6% (bezogen auf den Mehlanteil) betragen.

Das erfindungsgemässe biologische Backmittel ist zur Verbesserung aller durch mikrobiologische Vorgänge gelockerten Teige geeignet:

Weizenbrote, Weizensüssteige und Weizenmischbrote (Roggenmehlanteil bis 40%): Als Vorteigersatz (Hebel), mit Zugabe von Enzymen, Malzmehl und/oder Ascorbinsäure oder Fruchtpulver anstelle von konventionellen Backmitteln, zur Verbesserung der Teiglockerung, zur Intensivierung des Brotaromas, zur Standardisierung der Brotqualität.

Roggenbrote und Roggenmischbrote (ab 40% Roggenmehlanteil): Als Starterkultur für den Sauerteig, zur Verbesserung der Teiglockerung, zur Intensivierung des Aromas, zur Standardisierung der Brotqualität.

Das Backmittel gemäss Erfindung ist dadurch gekennzeichnet, dass mindestens 1 Milchsäurebakterienstamm, vorzugsweise ein Gemisch aus mehreren Milchsäurebakterienarten, in einem durch enzymatische Hydrolyse aufgeschlossenen Getreiderohstoff, vorzugsweise aus biologisch-kontrolliertem Anbau, gezüchtet werden. Die Hydrolyse der Getreiderohstoffe, welche vorzugsweise Weizenmehl und Weizenkeime sind, erfolgt durch mindestens 1 Amylase sowie mindestens 1 Amyloglucosidase und mindestens zwei Proteasen, wovon eine Protease überwiegend endoproteolytische Eigenschaften aufweist. Als weitere Stickstoffquelle kann ein, mit ausschliesslich physikalischen Methoden hergestellter, Bierhefeextrakt eingesetzt werden. Das aufgeschlossene Nährmedium wird sterilisiert, und die Züchtung erfolgt unter Sterilbedingungen. Dem Nährmedium und damit dem Backmittel werden keine chemischen Nähr- oder Hilfsstoffe zugesetzt, insbesondere erfolgt auch keine Kontrolle des pH-Wertes.

Die Herstellung des Nährmediums ist von entscheidender Bedeutung für den Geschmack des Backmittels und damit der daraus hergestellten Brote.

Das erfindungsgemässe Verfahren ist weiterhin dadurch gekennzeichnet, dass keine Konzentrierung oder Haltbarmachung erfolgt. Das Medium wird mit allen Aromakomponenten und den lebenden Lactobazillen den Teigen zugegeben. Die Haltbarkeit bis zu 6 Wochen wird durch Kühllagerung erreicht. Zur Verhinderung der Sedimentation können der Lösung noch Hydrokolloide wie in nachfolgender, nicht abschliessender Liste, genannt, zugeführt werden (niederverestertes Pektin + Ca-Salz, Quellmehle, Guarmehl).

Vorzugsweise benuzt werden Milchsäurebakterien der Arten Lactobazillus, Leuconostoc und/oder Pediococcus, vorzugsweise einem Stamm Lactobazillus brevis, Lactobazillus plantarum, Leuconostoc mesenteroides und/oder Pediococcus pentosaceus und speziell eine oder mehrere Stämme von Lactobazillus brevis DSM 9209, Lactobazillus

plantarum DSM 9208, Leuconostoc mesenteroides DSM 9207, und Pediococcus pentosaceus DSM 9210 benutzt. Diese Stämme wurden aus Weizensauerteig isoliert. Die Merkmale der genannten Stämme sind in den Tabellen 1a und 1b zusammengefasst.

Beispiele;

1. Zubereitung eines geeigneten Nährmediums für die Milchsäurebakterien

In einem Bioreaktor, der 15 Liter fasst, mischt man 9 Liter Wasser, 600 g Bio-Weizenkeime, 380 g gemahlene Bio-Weizenkörner, 20 g Bierhefeextrakt, 1 ml Alpha-Amylase-Lösung (16 Einheiten RAU/gramm) zur Hydrolyse der Stärke. Die Mischung wird während 20 Minuten auf 75°C gehalten und anschliessend auf 55°C abgekühlt. Man fügt 6 ml Amyloglucosidase-Lösung (17,6 AGI/g), 0,12 ml multi-aktives β-Glucanase Präparat (45 FBG/g), 2 ml Endoprotease (2,4 AU/g) und 3 ml Exopeptidase (800 LAPU/g) dazu. Die Tätigkeit der Enzyme dauert 120 Minuten. Das erhaltene Nährmedium wird während 20 Minuten bei 120°C sterilisiert. Dieses Medium wird bei 4°C gelagert und zur Fermentation der Milchsäurebakterien eingesetzt.
Die freigesetzten Zucker werden durch eine leistungsstarke HPLC analysiert. Die durch Hydrolyse der Proteine freigesetzten Aminosäuren werden durch die Reaktion mit Ninhydrin analysiert (S. Moore und W.H. Stein, J. Biol. Chem. 176,367,1948).

Die erhaltenen mittleren Werte sind:

Maltose ca.	8 g/l
Glucose ca.	50 g/l
Aminosäuren ca.	15 g/l

Zu keinem Zeitpunkt der Zubereitung des Mediums werden chemische Zusätze zugefügt. Der pH-Wert des Mediums liegt bei 6,0; z. B. 5,5 - 6,5.

2. Fermentation der Mikroorganismen

Die für ihren Stoffwechsel und für ihre sensorischen Eigenschaften ausgewählten Milchsäurebakterien Stämme werden bei - 80°C konserviert. Bei der Benutzung werden sie auf festes Getreidemedium umgesetzt (vorher beschriebenes Nährmedium 15 g/l Bactoagar).
Zwei Kulturen werden successive im Nährmedium gezüchtet. Die erste Kultur wird anhand einer isolierten Kolonie in 100 ml Medium angeimpft, (24 Stunden kultiviert bei 30°C ungeschüttelt). Die zweite Kultur wird in 900 ml neues Nährmedium umgesetzt, das mit 100 ml der vorhergehenden Kultur beimpft wird (24 Stunden kultiviert bei 30 °C ungeschüttelt). Diese Kultur dient dazu, zu der gewünschten Zeit und in der gewünschten Konzentration den Bioreaktor zu beimpfen.

Die Eigenschaften der benutzten Milchsäurebakterien Stämme in reiner Kultur im Nährmedium sind in der Tabelle 2a aufgezeigt. Einige Beispiele von gemischter oder sequenziell gemischter Kultur sind in den Tabellen 2b und 2c erfasst. Diese Beispiele sind ein Kompromiss, der als Ziel hat, genügend angeimpfte Milchsäurebakterien zu produzieren und in dem Brot ein charakteristisches Aroma und einen typischen Säuregehalt zu erhalten. Die Beimpfung mit Milchsäurebakterien in gewünschter Zeit und gewünschter Menge erlaubt die Konzentration der verschiedenen Mikroorganismen zu regulieren und somit die sensorischen Eigenschaften des Endprodukts zu beeinflussen. (Beispiele: siehe Tabellen 2b und 2c).

Tabelle 1a: *Morphologische und physiologische Merkmale der Milchsäurebakterien*

	L. Plantarum DSM 9208	*L. brevis* DSM 9209	*L. mesenteroides* DSM 9207	*P. pentosaceus* DSM 9210
Kolonie-merkmale (2 Tage, MRS Agar)	Koloniedurchmesser > 1 mm weiss smooth	Koloniedurchmesser > 1 mm grau smooth	Koloniedurchmesser 0,5 - 1 mm grau smooth	Koloniedurchmesser > 1 mm weiss smooth
Zellformen und Länge MRS Bouillon	Stäbchen verschiedene Länge einzeln bis Ketten	Stäbchen verschiedene Länge einzeln bis Ketten	Kokken bis Kurzstäbchen	Kokken, in Tetraden auch zu Paaren
Milchsäurekonfiguration	DL	DL	D	DL
Wachstum bei 15°C Wachstum bei 45°C	+ +	+ +/-	+ -	+ +
End pH in MRS Bouillon	3,4	4,4	4,2	3,7
Gasbildung aus Glucose Ammoniak aus Arginin Diaminopimelinsäure	- - +	+ + -	+ - -	- + -

+ = Reaktion positiv +/- = Reaktion schwach - = Reaktion negativ

Tabelle 1b: Zuckervergärungsspektren der Milchsäurebakterien (Api 50 CH Biomérieux)

	L. plantarum DSM 9208	*L. brevis* DSM 9209	*L. mesenteroides* DSM 9207	*P. pentosaceus* DSM 9210
Contrôle	-	-	-	-
Glycérol	-	-	-	-
Erythritol	-	-	-	-
D-Arabinose	-	-	-	-
L-Arabinose	+	+	+	+
Ribose	+	+	+	+
D-Xylose	-	+	+	-
L-Xylose	-	-	-	-
Adonitol	-	-	-	-
beta-Methyl-xyloside	-	+	-	-
Galactose	+	+	+	+
D-Glucose	+	+	+	+
D-Fructose	+	+/-	+	+
D-Mannose	+	-	+	+
L-Sorbose	-	-	-	-
Rhamnose	+/-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
Mannitol	+	-	-	-
Sorbitol	+	-	-	-
alpha-Methyl-D-mannoside	+	-	-	-
alpha-Methyl-D-glucoside	+	+	+	-
N-Acetyl-glucosamine	+	+/-	+	+
Amygdaline	+	-	+	-
Arbutine	+	-	+	+
Esculine	+	-	+	+
Salicine	+	-	+	+
Cellobiose	+	-	+	+
Maltose	+	+	+	+
Lactose	+	-	-	-
Mélobiose	+	+/-	+	-
Saccharose	+	-	+	-
Tréhalose	+	-	+	-
Inuline	-	-	-	-
Mélézitose	+	-	-	-
D-Raffinose	+	-	+	-
Amidon	-	-	-	-
Glycogène	-	-	-	-
Xylitol	-	-	-	-
beta-Gentibiose	+/-	-	+/-	+
D-Turanose	+	-	+	-
D-Lyxose	-	-	-	-
D-Tagatose	-	-	-	+
D-Fucose	-	-	-	-
L-Fucose	-	-	-	-
D-Arabitol	+/-	-	-	-
L-Arabitol	-	-	-	-
Gluconate	+/-	+/-	-	-
2-Céto-gluconate	-	-	-	-
5-Céto-gluconate	-	+/-	-	-

Tabelle 2a: Fermentation der Milchsäurebakterien alleine

Erste Kultur 1 Oese (100 ml Nährmedium) - 24 Stunden - 30°C - ohne Schütteln

Zweite Kultur Verdünnung der ersten Kultur zu 1/10 - 16 Stunden - 30°C - leicht Schütteln

Zweite Kultur	*L. plantarum* DSM 9208	*L. brevis* DSM 9209	*L. mesenteroides* DSM 9207	P. pentosaceus DSM 9210
Zellzahl (x 10^9 zellen/ml)	2,5	4,0	1,7	2,1
pH	3,8	3,95	4,02	3,85
Glucose (g/l)	28,5	27,2	26,3	26,7
Milchsäure (g/l)	10,5	6,8	6,5	11,1
Essigsäure (g/l)	0	2,9	1,1	0
Ethanol	0	0,7	2,4	0

Tabelle 2b: Fermentation der Milchsäurebakterien gemischt

Erste Kultur 1 Oese (100 ml Nährmedium) - 24 Stunden - 30°C - ohne Schütteln

Zweite Kultur Verdünnung der ersten Kultur zu 1/20 - 16 Stunden - 30°C - leicht Schütteln

Zweite Kultur		*L. plantarum* DSM 9208 und *L. brevis* DSM 9209	*L. plantarum* DSM 9208 und *L. mesenteroides* DSM 9207	*P. pentosaceus* DSM 9210 und *L. mesenteroides* DSM 9207	*L. brevis* DSM 9209 *L. mesenteroides* DSM 9207 *L. plantarum* DSM 9208
Zell-zahl ($\times 10^9$)	*L. brevis* DSM 9209	4,0	0	0	1,6
	L. plantarum DSM 9208	1,2	3,0	0	2,5
	L. mesenteroides DSM 9207	0	0,1	0,5	0,4
	P. pentosaceus DSM 9210	0	0	2,0	0
pH		3,78	3,73	3,80	3,85
Glucose		23,0	24,1	23,5	25,0
Milchsäure		13,4	12,5	11,4	11,9
Essigsäure		5,3	0,3	0,2	1,4
Ethanol		2,1	0,9	0,6	0,6

Tabelle 2c: Fermentation der Milchsäurebakterien sequentiell gemischt

Erste Kultur 1 Oese (100 ml Nährmedium) - 24 Stunden - 30°C - ohne Schütteln

Zweite Kultur Verdünnung der ersten Kultur - 16 Stunden - 30°C - leicht Schütteln

Zweite Kultur		*L. mesenteroides* DSM 9207 verdünnung 1/10 *L. plantarum* DSM 9208 verdünnung 1/20 nach 4 St	*L. plantarum* DSM 9208 verdünnung 1/20 *L. brevis* DSM 9209 verdünnung 1/20 nach 4 St	*L. plantarum* DSM 9208 verdünnung 1/10 *L. brevis* DSM 9209 verdünnung 1/20	*L. plantarum* DSM 9208 und *L. brevis* DSM 9209 verdünnung 1/50
Zell-zahl (x 10^9)	*L.brevis* DSM 9209	0	2,4	1,0	3,4
	L.plantarum DSM 9208	2,4	2,7	3,0	1,7
	L. mesenteroides DSM 9207	1,0	0	0	0
	P. pentosaceus DSM 9210	0	0	0	0
pH		3,80	3,75	3,73	3,77
Glucose		25,2	26,1	24,3	27,3
Milchsäure		10,8	12,5	16,2	11,3
Essigsäure		0,5	4,0	2,7	4,3
Ethanol		1,2	3,7	0,9	2,1

Patentansprüche

1. Verfahren zur Herstellung eines flüssigen oder pastösen biologischen Backmittels, dadurch gekennzeichnet, dass Getreiderohstoffe und Bierhefeextrakt gemischt werden und mindestens eine Amylase und mindestens eine Amyloglucosidase zugegeben wird, wobei das Medium zuerst 10 - 30 Minuten auf 50 - 80°C aufgeheizt, dann auf 55°C abgekühlt und nach Zugabe von mindestens 2 Proteasen 30 Minuten - 4 Stunden bei dieser Temperatur gehalten wird, woran sich dann die Sterilisation und Fermentation zu dem Backmittel mit Hilfe von einer oder mehrerer Milchsäurebaktereienarten anschliesst.

2. Verfahren gemäss Anspruch 1, gekennzeichnet dadurch, dass die Züchtung der Milchsäurebakterien-Kulturen diskontinuierlich durchgeführt werden.

3. Verfahren nach einem der Ansprüche 1 bis 2, gekennzeichnet dadurch, dass die Züchtung der Milchsäurebakterienarten ohne pH-Regelung durchgeführt wird.

4. Verfahren nach einem der Ansprüche 1 bis 3, gekennzeichnet dadurch, dass die eingesetzten Milchsäurebakterien solche der Arten Lactobazillus, Leuconostoc und/oder Pediococcus sind.

5. Verfahren nach einem der Ansprüche 1 bis 4, gekennzeichnet dadurch, dass die eingesetzten Milchsäurebakterien solche der Stämme Lactobazillus brevis, Lactobazillus plantarum, Leuconostoc mesenteroides und/oder Pediococcus pentosaceus sind.

6. Verfahren nach einem der Ansprüche 1 bis 5, gekennzeichnet dadurch, dass die eingesetzten Milchsäurebakterien aus einem oder mehreren Stämmen Lactobazillus brevis die DSM 9209, Lactobazillzus plantarum DSM 9208, Leuconostoc mesenteroides DSM 9207 und Pediococcus pentosaceus DSM 9210 sind.

7. Verfahren nach einem der Ansprüche 1 bis 6, gekennzeichnet dadurch, dass es sich bei den Getreiderohstoffen um Keime und Mehle, gegebenenfalls in Mischungen, handelt.

8. Verfahren nach einem der Ansprüche 1 bis 7, gekennzeichnet dadurch, dass das Medium zuerst 20 Minuten auf 75°C aufgeheizt und dann auf 55°C und nach Zugabe von mindestens 2 Proteasen 2 Stunden bei dieser Temperatur gehalten wird.

9. Benutzung der gemäss einem der Ansprüche 1 bis 8 erhaltenen biologisch-aktiven Lösungen als Backmittel vorzugsweise als Vorteig oder Starterkultur für die Brotherstellung.

10. Das vorzugweise biologische Backmittel gemäss den Ansprüchen 1 bis 9 enthält ein oder mehrere stärkeabbauende Enzyme (Amylasen).

11. Das Backmittel gemäss der Ansprüche 1 bis 10 enthält das Enzym Glucoseoxidase.

12. Das Backmittel gemäss den Ansprüchen 1 bis 11 wird dadurch gekennzeichnet, dass Ascorbinsäure oder ascorbinsäurehaltiges Fruchtpulver zugesetzt werden.

13. Verfahren nach den Ansprüchen 1 bis 12, dadurch gekennzeichnet, dass eine Dosierung von 1 bis 10% vorzugsweise von 2 bis 6% (bezogen auf den Mehlanteil), erfolgt.

14. Das Backmittel, gemäss den Ansprüchen 1 bis 13, wird dadurch gekennzeichnet, dass enzymaktive oder enzyminaktive Malzmehle oder Malzextrakte zugesetzt werden können.